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Inhibitory effects of arabitol on caries-associated microbiologic parameters of oral Streptococci and Lactobacilli

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ABSTRACT

The aim of this study was to compare arabitol with its better studied isomer xylitol for their inhibitory effects on cell growth and acid production of oral bacteria. *Streptococcus mutans, Streptococcus salivarius* and *Streptococcus sobrinus* were used as representatives of oral streptococci and *Lactobacillus acidophilus* and *Lactobacillus fermentum* were used for oral lactobacilli. Growth was followed by measuring the absorbance at 660 nm, acid production by pH change. Sensitivity of these oral bacteria to arabitol and xylitol was first compared at 1% (65 mM) additive concentration with glucose as sugar substrate. For all bacteria tested, the inhibitory effects of the two polyols were comparable; both were significantly stronger on streptococci (with 20–60% inhibition) than on lactobacilli (with 5–10% inhibition). Effects of arabitol and xylitol were also compared for *S. mutans* and *S. salivarius* in media with 1% of different sugar substrates: glucose (55 mM), fructose (55 mM), galactose (55 mM) and sucrose (30 mM). Inhibition occurred for all sugars: stronger on glucose and galactose (60–65%) than on fructose and sucrose (40–45%). Inhibition dependency on the arabitol/xylitol concentration from 0.01% (0.65 mM) to 2% (130 mM) was further determined for *S. mutans* and *S. salivarius*. Regardless of the concentration, sugar substrate and bacterial species tested, arabitol showed very similar inhibition effects to its isomer xylitol.

1. Introduction

Dental caries is one of the most prevalent chronic infectious disease in the world (Anusavice, 2002). Although the widespread presence of dental caries has been reduced significantly over last few decades in some countries, the disease is still a major problem for both adults and children. Among the main factors causing dental caries are the metabolic activities of cariogenic microorganisms. Mouth contains a wide variety of bacteria; some of them are believed to cause dental caries: *Streptococcus mutans, Streptococcus salivarius*, and some lactobacilli are among them. There are many reports on which microorganisms are more dangerous for causing dental caries. Particularly for root caries and cavity formation in children's teeth, *S. mutans* and *Lactobacillus acidophilus* are frequently identified as the most closely related bacteria (Becker et al., 2002). These bacteria are found around the teeth and gums in a sticky, creamy-colored mass called plaque, which serves as an adhering biofilm. These bacteria are necessary but not sufficient for developing dental caries, which is exacerbated by different dietary factors (Kleinberg, 2002; Van Houte, 1994). Excessive consumption of sugars, especially sucrose and glucose, is proved to be most responsible for the prevalence of dental caries regardless of the geography or age (Newbrun, 1982; Tinanoff & Palmer, 2000; Touger-Decker & Van Loveren, 2003). When fermentable carbohydrates such as glucose, sucrose, fructose and galactose are taken into mouth, they are metabolized by these bacteria with production of organic acids such as lactic acid, acetic acid and formic acid. These acids diffuse through the plaque and into the porous enamel, dissolving the minerals, freeing calcium and phosphate into the solution (i.e., demineralization). The weakened enamel then collapses to form a cavity and the tooth is progressively destroyed (Fozo & Quivey, 2004; Marsh, 2006; Paes Leme et al., 2006).

This damaging acid production from fermentable carbohydrates has prompted the search for an easily available and acceptable sugar substitute. Xylitol is well-known for the anticariogenic property (Mäkinen, 2010). It is not fermented by the cariogenic bacteria such as *S. mutans* and *Streptococcus sobrinus* (Havenaar, 1984; Lynch & Milgrom, 2003). It also inhibits the acid production that would cause pH decrease in dental plaque and enamel demineralization. Chewing gums containing xylitol have been reported to reduce the dental plaque and the number of *S. mutans* in saliva (Aguirre-Zero, Zero, & Proskin, 1993; Mäkinen, 2011). Xylitol is currently produced by chemical reduction of

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xylose derived from wood hydrolysate under alkaline condition. But purification and separation of xylose as well as the required high pressure and temperature and expensive catalyst make the process less economical (Nigam & Singh, 1995; Winkelhausen & Kuzmanova, 1998). Arabitol, a five carbon polyhydroxy sugar alcohol, is a 2′-epimer of xylitol. Arabitol however has never been investigated for its potential to inhibit the oral bacteria as xylitol. Moreover, sweetness per caloric content for arabitol is much higher than that of xylitol (Huck, Roos, Jakobs, Van der Knaap, & Verhoeven, 2004; Lee, 1977; McCormick & Touster, 1961). Sweetness vary among sucrose and different sugar alcohols. The degree of sweetness may vary from about half as sweet as the same amount of sucrose to equally as sweet as sucrose (Table 1).

Compared to sucrose, sugar alcohols are slowly and incompletely absorbed and metabolized in the small intestine which makes their caloric contents lower than sucrose. Among the sugar alcohols, arabitol and erythritol were reported to have very low glycemic responses and very low caloric contents as compared to other sugars. In addition to its low sweetness per caloric content, arabitol, like other sugar alcohols, can produce a noticeable cooling sensation in the mouth when consumed, due to the dissolution of arabitol being an endothermic reaction (Lee, 1977). Arabitol can be produced by osmophilic yeasts using sugars (e.g. glucose, arabinose) as substrate (Escalante, Caminal, Figueredo, & de Mas, 1990; Saha, Sakakibara, & Cotta, 2007). Recently, production of arabitol from glycerol, a biodiesel byproduct, by *Debaryomyces hansenii* 23 and subsequent purification process have been reported (Loman & Ju, 2013). These processes may make both arabitol and biodiesel production more economical.

The objective of this study was to compare the purified arabitol with commercial xylitol in terms of the inhibitory effects on growth and acid production of oral bacteria. First we investigated the effect of xylitol and arabitol, at a fixed 1% concentration, on 5 oral bacteria *S. mutans, S. salivarius, S. sobrinus, L. acidophilus* and *Lactobacillus fermentum*. Then the species found to be more inhibited by xylitol and arabitol, were further investigated for the inhibition effects in presence of different sugar substrates. Effects of different arabitol/xylitol concentrations were also investigated.

2. Materials and methods

2.1. Microorganisms

S. mutans (ATCC 10449), *S. salivarius* (NRRL B-3714), *S. sobrinus* QMZ 176 (NRRL B-4468), *L. acidophilus* (NRRL B-4495), and *L. fermentum* (NRRL B-1840) were used in this study. *S. mutans* was purchased from American Type Culture Collection (ATCC). The other four cultures were provided by USDA Agricultural Research Service (ARS) Culture Collection (Peoria, IL). The bacteria were maintained by weekly transfers on agar plates of trypticase yeast extract medium (Sigma–Aldrich, St. Louis, MO) containing 0.5% glucose and 1.5% agar.

2.2. Chemicals

Arabitol used in the experiment was produced in the laboratory by *D. hansenii* fermentation as described elsewhere (Koganti, Kuo, Kurtzman, Smith, & Ju, 2011). Arabitol obtained from fermentation broth was purified through the process of activated carbon treatment followed by vacuum concentration, acetone extraction of glycerol, butanol extraction, and crystallization, which yielded at least 95% pure arabitol (Loman & Ju, 2013). Xylitol (99+%) was purchased from Sigma–Aldrich (St. Louis, MO).

2.3. Inhibitory effect experiments on 5 bacterial species

All five strains of bacteria were cultured in the Brain Heart Infusion (BHI) Medium (Sigma–Aldrich, St. Louis, MO) containing (in 1 l solution) 17.5 g Calf Brain–Beef Heart Infusion, 10 g pancreatic digest of gelatin, 3 g NaCl, 2.5 g dipotassium phosphate (K_2HPO_4), 1 g monopotassium phosphate (KH_2PO_4) and 10 g glucose (Fisher Scientific, Hampton, NH). Xylitol and arabitol solutions were autoclaved separately and added to the sterile BHI medium to give 1% concentration. Control systems were prepared using same medium without any xylitol or arabitol. Cells were grown in 25 ml medium in a 125 ml Erlenmeyer flask inoculated with 5% (v/v) seed culture. (The seed culture was prepared by inoculating a single colony of cells from an agar plate into 25 ml BHI medium and incubating it overnight at 37 °C anaerobically). Cultures were flushed with N_2 gas to make them anaerobic at the beginning and each time after collection of sample and closed by rubber stopper and incubated at 37 °C for 36 h in a shaker (Thermo Scientific MaxQ 5000 Incubating/Refrigerating Floor Shaker, Asheville, NC) at 250 rpm. Samples were taken periodically for analysis.

2.4. Effect of different sugar substrates

From the results of growth and acid production by all 5 bacteria, two of them, *S. mutans* and *S. salivarius*, were selected for investigating the effects of 4 different sugar substrates (glucose, galactose, fructose, sucrose) with or without 1% arabitol or 1% xylitol on the growth and acid production of these bacteria. In these experiments, for each bacterium studied, there were three systems for each substrate (e.g. 1% fructose): one control (without arabitol or xylitol), one with 1% arabitol, and another with 1% xylitol.

2.5. Effect of arabitol or xylitol concentration

S. mutans and *S. salivarius* were further investigated for the comparative effect of different concentrations of xylitol and

Table 1
Comparison of sweetness and caloric content for different sugar substitutes, with sucrose as reference for sweetness (Belitz, Grosch, & Schieberle, 2009; Mitchell, 2008).

Name	Relative sweetness (sucrose = 1.0)	Caloric content (kcal/g)	Sweetness per caloric content
Arabitol	0.7	0.2	3.5
Erythritol	0.8	0.2	3.5
Maltitol	0.9	2.1	0.43
Xylitol	1.0	2.4	0.42
Mannitol	0.5	1.6	0.31
HSH[a]	0.4–0.9	3.0	0.13–0.3
Isomalt	0.5	2.0	0.25
Sorbitol	0.6	2.6	0.23
Lactitol	0.4	2.0	0.2
Glycerol	0.6	4.3	0.14
Sucrose	1.0	4.0	0.25

[a] HSH: hydrogenated starch hydrolysate.